SureSc re

Contents:

Product Name And Cat. No.:

SureScore. A/G Genotyping Kit	T6000-01
SureScore. T/C Genotyping Kit	T6000-02
SureScore. A/C Genotyping Kit	T6000-03
SureScore. T/A Genotyping Kit	T6000-04
SureScore. G/C Genotyping Kit	T6000-05
SureScore. T/G Genotyping Kit	T6000-06
SureScore. Complete Genotyping Kit	T6000-07

SNPwareTM 96, Core Reagent Kit, Part # 101-0030-01
SNPwareTM 96, A/C Extension Reagent Kit, Part # 101-0023-01
SNPwareTM 96, A/G Extension Reagent Kit, Part # 101-0024-01
SNPwareTM 96, G/C Extension Reagent Kit, Part # 101-0025-01
SNPwareTM 96, T/A Extension Reagent Kit, Part # 101-0026-01
SNPwareTM 96, T/C Extension Reagent Kit, Part # 101-0028-01
SNPwareTM 96, T/G Extension Reagent Kit, Part # 101-0029-01

Description:

99% of the chemical structure of everyone's DNA is the same. On a genome scale, however, there are numerous specific sequence differences that identify individuals. Single nucleotide polymorphisms are rare mutation. These specific sequences are called single nucleotide polymorphisms (SNPs, pronounced "SNiPs"). There is 1 SNP every 1000bp (approx) or 60,000 SNPs in coding region. In the MHC 1 SNP every 100bp, so it varies.
Single nucleotide polymorphisms (SNPs) are a common form of genomic variation distinguished from rare mutations by having the least abundant allele at a frequency > 1% in a given population. The risks of major common disease such as cancer, cardiovascular disease, mental illness, autoimmune states and diabetes are expected to be heavily influenced by the patterns of SNPs in key susceptibility genes. The single base extension approach originally described as Genetic Bit Analysis (GBA) relies upon the inherent ability of DNA polymerase to distinguish SNPs. It is an established, simple and robust method for routine genotyping. Orchid has produced a low throughput kit in 96 well format called SNP-IT (or SNPware 96) based on the GBA technology.

We estimate that the SureScore kit has a 99% accuracy.

Applications:

In cases where one must quickly move from one set of markers to the next and develop the assays along the way, methods that require little or no optimization are the most appropriate for SNP scoring. This is a manual assay designed for low throughput customers.

1) To identify single nucleotide mutations that are responsible for drug responsivity, cancer, and other disease
2) Haplotype mapping (see literature reference)

Components:

Module I-Core Reagent Kit	**Part #**
20X Wash Buffer (200 ml)	200-0002-12
Attachment Buffer (38 ml)	200-0002-13
TargEx Buffer (10 ml)	200-0002-14
Hybridization Solution (10 ml)	200-0002-15
Extension Dilution Buffer (18 ml)	200-0002-16
Detection Complex Dilution Buffer (40 ml)	200-0002-17
Detection Complex I (60 µl)	200-0002-18

Detection Substrate I (60 ml)	200-0002-19
Detection Complex II (60 µl)	200-0002-20
Detection Substrate II (60 ml)	200-0002-21
Product Manual	950-
*96 strip-well SNP-IT plates (5)	750-0056-61 (can mention that the plates are from Orchid and the customer can contact them about individual plates.
Product Insert	101-0030-01

Module II-Extension Reagent Kits
TargEx Enzyme (36 µl)
10X Extension Mix (360 µl)
Control Primer (12 µl)
Control Template XX (200 µl)
Control Template YY (200 µl)
Control Template XY (200 µl)
Product Insert

Extension Reagent Kit Selection Guide

The type of extension mix for each SNPware 96 Extension Reagent Kit indicates which bases are present as labeled terminating nucleotides. These terminating nucleotides are subsequently incorporated into the SNP-IT assay using DNA polymerase and serve as the basis for the SNP site interrogation.

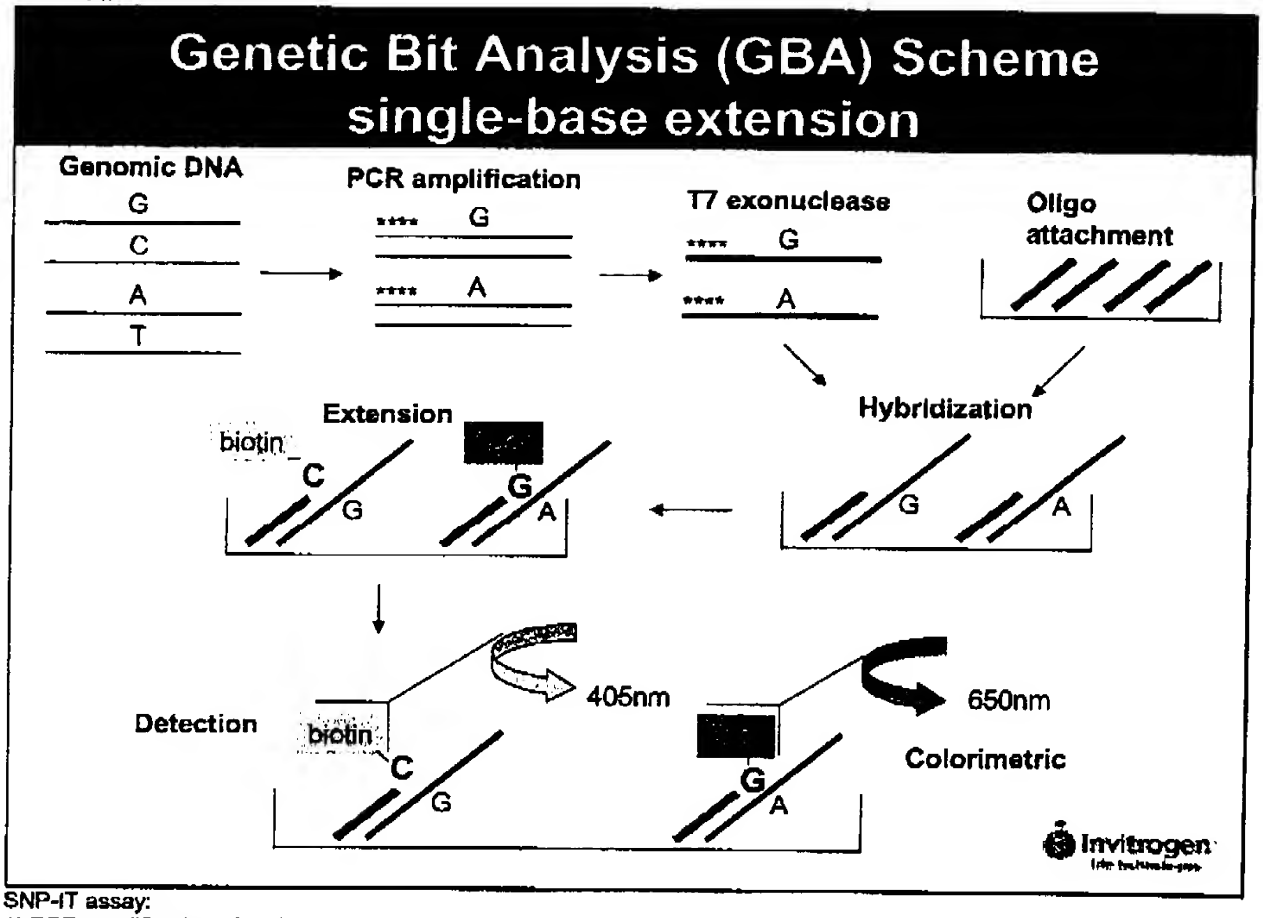
Bases Involved in SNP Sequence of Interest (5'– 3')	SNP-IT Primer Recommended By Autoprimer. com	Recommended SNPware 96 Kit
A/G	SNPU	SNPware 96, A/G kit
A/G	SNPL	SNPware 96, T/C kit
T/C	SNPU	SNPware 96, T/C kit
T/C	SNPL	SNPware 96, A/G kit
A/C	SNPU	SNPware 96, A/C kit
A/C	SNPL	SNPware 96, T/G kit
T/A	SNPU	SNPware 96, T/A kit
T/A	SNPL	SNPware 96, T/A kit
G/C	SNPU	SNPware 96, G/C kit
G/C	SNPL	SNPware 96, G/C kit
T/G	SNPU	SNPware 96, T/G kit
T/G	SNPL	SNPware 96, A/C kit

U=Upper Strand Primer
L=Lower Strand Primer

The SNPwareTM 96 reagent kits consist one core reagent kit and six extension specific kits:
SNPwareTM 96, Core Reagent Kit, Part # 101-0030-01
SNPwareTM 96, A/C Extension Reagent Kit, Part # 101-0023-01
SNPwareTM 96, A/G Extension Reagent Kit, Part # 101-0024-01
SNPwareTM 96, G/C Extension Reagent Kit, Part # 101-0025-01
SNPwareTM 96, T/A Extension Reagent Kit, Part # 101-0026-01
SNPwareTM 96, T/C Extension Reagent Kit, Part # 101-0028-01
SNPwareTM 96, T/G Extension Reagent Kit, Part # 101-0029-01
The core reagent kit contains five 96 strip-well plates and common 4oC storage reagents necessary for performing 480 SNP-IT assays.
Each extension specific kit contains perishable enzymes, 10X Extension Mix and Controls are specified for each kit based on the extension type. Each of the six kits provides enough extension specific reagents that can be used for 96 SNP-IT assays. These reagents need to be stored at –20°C.

Shipping/Stability/Storage:
Module I-Core Reagent Kit,
All items should be stored at 4°C. However, the Product Manual and the 96 Strip-well SNP-IT Plates may be stored at room temperature upon receipt.
Module II-Extension Reagent Kits
All items should be stored at -20°C. The 10X Extension Mix and Control Templates will be unique to each kit type provided.

Procedures:



SNP-IT assay:
1) PCR amplification of region surrounding the SNP of interest from a genomic DNA sample.
2) Generation of a single-stranded target sequence by exonuclease digestion of the PCR amplified product containing the target SNP.
3) The single-stranded target sequence is hybridized to a SNP-IT primer that is attached to a 96-well plate. The immobilized target strand will serve as a template for primer extension.
4) Extension of the primer by labeled terminating nucleotides and DNA polymerase.
5) Detection of the incorporated terminator in an colorimetric assay.

PCR Conditions:

Special Protocol Note:
There is a shorter version, however, the overall robustness of the shorten protocol was about half that of the standard protocol.
The shorter version included:

1) reducing the incubation time of the GBA oligo to the 96 well plate from overnight incubation to1 hour
2) reducing exonuclease digestion from 1 hour at room temp to 10 minutes at 37C
3) reducing hybridization from 1 hour at room temp to 15 minutes at 37C
4) combining both antibodies in one 30 minute incubation instead of two separate 30 minutes incubations plus six washes.

5) The shortened protocol reduces the assay time from "O/N incubation plus 5.5 hours" to roughly a "1 hour incubation plus 3.5 hours".

The short protocol has only been performed with a limited number of robust SNPs. It is possible that this protocol is only applicable for a limited number of robust SNPs since there is on average an 11% (3-25%) reduction in overall signal intensity.

Note:

Most current SNP scoring methods use one of four methods for allelic discrimination

1) allele-specific hybridization
2) allele-specific primer extension
3) allele-specific oligonucleotide ligation
4) allele-specific cleavage. These methods are used in combination with one of the following types of detection systems
    a. Colorimetric ELISA
    b. Fluorescent intensity
    c. Fluorescent polarization
    d. Mass spectrometry for SNP scoring.

5) Specify four phosphorothioates on the 5' end of the primer during ordering.

Troubleshooting:

- The SNP96 ware lower limit assay parameters require approximately 2ng of genomic DNA to generate a PCR product at 35 cycles. The assay requires approximately 0.1ng of capture DNA and ~50ng of PCR product to obtain a scorable genotype.
- The PCR fragment size must be between 100-150bp. Fragments greater than 180bp will result in decreased signal. However, the above ranges are not absolute and will vary depending on the SNP.
- Controls not working well e.g. the positive control for A sometimes showing A and at times showing G. According to Pat Gilles, this could be due to: (1) high concentration of control capture at the time of attaching to plate. Solution: Customer should check and ensure they are doing the attaching at 0.0625uM (they may be doing it the wrong conc of 0.25uM). In this case they should also be seeing high background with the G because the capture conc. is too high there as well. (2) The template was not washed away due to omission of NaOH or step not being done properly. If the template is still there it too will get extended and will generate a competing signal. (3) Incomplete wash washing after incubation eith the conjugated antibodies. Solution: Do Two extra washes after the antibody incubation and thoroughly remove all the solution after each wash.

Frequently Asked Questions:

1. How much DNA template do I need in the PCR reaction?

As an initial starting point, we recommend using between 0.1 and 1ng of template DNA per µl of each individual PCR reaction. This is also dependent on the number of PCR cycles. At Orchid, we have observed that 1 ng/µl for 36 cycles, works well in the majority of cases. However, it is important to note that the concentration of template DNA that is necessary for successful PCR can vary widely depending on the specific PCR primers utilized and the target DNA sequence being amplified. Additionally, it is also very important that the purity of the template DNA is good. We strongly recommend using DNA with an A260/A280 ratio between 1.7-2.0. This DNA should also be devoid of any potential PCR inhibitors.

2. Should I run the assay if I do not see a PCR band on the gel?

It is always prudent before proceeding to the SNP-IT assay to ensure that the appropriate sized PCR amplified product is present. Therefore, it is always preferable that the target DNA fragment is able to be visualized by gel electrophoresis. In those cases where no PCR product can be visualized, it is very important to confirm (through the use of appropriate controls) that the PCR reactions themselves did not fail. We have observed in some cases, where no PCR product was visualized by electrophoresis (but proper controls indicated that the PCR reactions were successful) that the PCR reactions were still capable of working well in the SNP-IT assay. This observation indicates that some SNPs require very little sample input for the SNP-IT assay to be successful. If the decision is made to proceed with a PCR product that cannot be visualized prior to the SNP-IT assay, it is important to include another sample in the SNP-IT assay that you know should work as an additional positive control for your SNP-IT assay.

3. How long are the SNP-IT plates stable once the SNP-IT primers have been attached to the surface?

It is best to use fresh SNP-IT plates for the assay. If needed, the plates can be stored at 4°C for up to a week after washing three times with Wash Buffer following the overnight incubation.

4. How long can I keep the plates after each colorimetric detection step before I actually analyze the results?
We highly recommend that each colorimetric detection step be analyzed for results immediately after the color development step to ensure that the most accurate genotype calls can be made. However, we have observed that the yellow reaction product is relatively stable and can be analyzed up to 2 hours post substrate incubation. The blue reaction product is much less stable and should be scored immediately without exception.

5. What is the optimum number of samples required for this assay?
We recommend using at least 20 samples for unconfirmed SNPs. This would provide sufficient data points to obtain a decent sized cluster during data analysis. This number can be drastically reduced, if internal sample controls (samples with known genotypes) are available and run in the assay. These samples can then be used as a reference for assigning genotypes to the unknown samples.

6. Should I be concerned if I observe three clusters in the scatter plot but one of them seems to be shifting to the other or are very close to each other?
OR
What should I do if my homozygous samples controls look like heterozygotes in the assay?
Both the above observations are usually due to template dependent noise. This happens when the SNP-IT primer anneals to more than one site on the PCR template, other than its intended location (immediately adjacent to the SNP of interest). This phenomenon leads to multiple extensions occurring at different sites and often produces a significant level of background in the SNP-IT assay. This is commonly observed as a shift towards heterozygotes in one or more of the genotype clusters. Since this is template specific, it is usually best to choose an alternate design for the assay primers.

7. What should I do if I see a split cluster on either axis?
This usually occurs if an alternate SNP site is present in the template, in the region complementary to the SNP-IT primer. This potential single base mismatch in some of the samples, at the alternate SNP site, may cause inefficient SNP-IT primer/template hybridization. Even though the extension step occurs, the signal is weaker in these samples and hence will show up as a distinct cluster in the scatter plot. This can be overcome by redesigning the SNP-IT for the opposite strand and on the other side of the SNP, so that the alternate SNP site is avoided altogether at the SNP-IT annealing step.

8. Is it possible to detect with the Sscore kit alleles present in percentages other than 50%? What kind of ratios? (10%: 90%?)
Roughly one can detect a variant allele at about 16% which is ~2-3 fold above background.

9. Is it possible to use the system with c-DNA and in RT-PCR?
Yes they can use cDNA or RNA via RT-PCR. They only requirement is that they use PCR primers to amplify a fragment of 80-180 bp which span the polymorphic site. They can visit our primer design web-site (www.invitrogen.com/surescore) where they put in their sequence of interest (w/SNP) and the software will give them primers (one primer has 4 phosphorthioates at the 5' end) to amplify the appropriate fragment and SNP-IT capture and select the appropriate extension mix for their sequence.

10. Is it possible to quantify one mutation compared to the other?
I don't know if one can quantitate allele frequency using this system - it may be possible - but an allele specific primer QC-PCR real-time format may be more applicable (P. Gilles 4/02)

11. Can this kit be used to detect insertions or deletions? How does it compare to RFLP?
Our kit can also identify insertions and deletions. The advantage of our kit, is that people can design any Oligo which is immediately adjacent to their polymorphic site of interest and our kit supplies all the reagents to determine which nucleotide is incorporated into the polymorphic site. If that site includes an insertion or a deletion, usually that can be determined by which nucleotide is incorporated. The problem with RFLP is that, if there isn't a restriction site on the polymorphism you're out of luck. The Surescore is flexible in that it can be designed for any SNP just two PCR primers (1

phosphothioated) and one SNP oligo for single-base extension. Our kit does not examine patterns it only scores single nucleotide sites. If both extension mixes are used our kit can also score mutations within a mixed population. (Patrick Gilles, 7/02)

Literature Available:
1) http://snp.cshl.org/
The SNP Consortium Ltd. is a 501c3 non-profit foundation organized for the purpose of providing public genomic data. Its mission is to develop up to 300,000 SNPs distributed evenly throughout the human genome and to make the information related to these SNPs available to the public without intellectual property restrictions. The project started in April 1999 and is anticipated to continue until the end of 2001.
2) Gura T. Genetics. Can SNPs deliver on susceptibility genes?
Science. 2001 Jul 27;293(5530):593-5.
PMID: 11474081

Licensing Info:
Orchid BioSciences, Inc. and Invitrogen entered into an agreement under which Invitrogen acquires exclusive worldwide rights to offer genotyping products to the research market utilizing Orchid's proprietary SNP scoring technology in a 96-well format. Under the terms of the agreement, Invitrogen can develop, manufacture and market genotyping kits using Orchid's proprietary SNP scoring technology and offer customers access to Orchid's proprietary SNP primer design algorithms. Invitrogen will pay Orchid a royalty on kit sales.
Orchid BioSciences, Inc. is a leading provider of products, services and technologies for single nucleotide polymorphism (SNP) scoring and genetic diversity analyses. Orchid has developed SNP-IT, its proprietary SNP analysis technology, and markets SNPstream© instruments and SNPware™ consumables that rapidly generate highly accurate, cost-effective SNP information. SNP-IT is usable in environments ranging from small-scale laboratories to large commercial facilities. The versatility of SNP-IT is enabling Orchid to partner with industry leaders to make SNP-IT-enabled products available on a wide variety of instrument platforms as part of Orchid's Platform Propagation™ strategy. Orchid also provides high throughput SNP scoring services to pharmaceutical, biotechnology, agricultural and academic customers through its MegaSNPatron™ facilities, and identity genomics testing for forensics and paternity, as well as clinical quality genotyping, through its GeneScreen and Cellmark facilities. Orchid also provides content-rich SNP databases and SNP panels to its customers and collaborators. Through its GeneShield business, Orchid seeks to identify and commercialize medical applications of SNPs. More information on Orchid can be found at its web site www.orchid.com.

Contacts:
R&D- Patrick Gilles
Manufacturing- Stephanie Roach
Product Manager- Frances Bai